On the Embryology of Stratiodrilus (Histriobdellidæ).

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With Plate 23 and 4 Text-figures.

The development of the Histriobdellidæ is practically unknown. In fact, so far as I am aware, the only statements regarding it which have been published are contained in P. J. van Beneden's original description of Histriobdella homari (**1**). These are very brief and superficial, and the figures given, except in so far as they show a process of complete segmentation and the absence of any metamorphosis, do not throw much light on the essentials of the development.

The eggs of Histriobdella homari were found by van Beneden attached separately to the membranous bands which unite together the eggs of the lobster. It is likely, however, that this is not the only situation in which they occur, but that they are also deposited in the branchial cavities.

In Stratiodrilus they occur mainly, if not exclusively, in the latter situation, and in S. novæ-hollandiæ, which I have chiefly studied, though now and then an odd one may be found attached to the base of a gill, the great bulk of them occur in the narrow exhalant passage leading forwards from the branchial cavity to open externally at the side of the mouth. In this position, attached to the inner surface of the narrow pointed extension of the branchial region of the carapace which bounds the passage in question externally, there are to be found in most specimens of *Astacopsis serratus* groups of the eggs, sometimes only a few, sometimes as many as a hundred, nearly always with a number of empty shells beside them. The eggs of *Histriobdella* are attached by one pole; those of *Stratiodrilus* by one side. The cementing material, in the latter case, is similar in appearance to the substance of the egg-shell, but reacts more readily to solvents such as hypochlorites.

The egg is about ·14 mm. in long, and ·10 mm. in short diameter. It is white, porcellanous, and very opaque, so that it is impossible to see much of the structure in the fresh condition. It is thus entirely out of the question to attempt to watch the development of the living embryo. Moreover, the shell, though not very thick, is extremely resistant, so much so that the making of preparations, whether of entire eggs or of sections, has proved a matter of exceptional difficulty. No satisfactory results could be obtained without the use of chitin-softening agents. Of these Henning's solution and soap-alcohol, after several trials, proved complete failures, and the only method by which results of any value were obtained was the following:

The area of cuticle bearing the batch of eggs was cut out and was subjected for some minutes to the action of hot (but not boiling) sublimate-acetic solution followed by distilled water. It was then placed in a weak solution of hypochlorite of soda ("liquor sodæ chlorinatæ" of the British Pharmacopœia 1 : water 25). The action of this had to be watched carefully. The rapidity of the effect is influenced in a marked degree by the temperature. The cementing material becomes softened first, so that the eggs may become detached by shaking or by touching with a camel's hair brush. When the softening process is judged to have gone on long enough, distilled water is substituted for the hypochlorite solution, and after a thorough washing is itself replaced by alcohol.

Since it is impossible to see anything of the structure of the egg except in the very earliest stages, definite orientation is impossible. I have found it of advantage, however, to have all the eggs cut in the same general direction; all may be cut longitudinally by their being allowed to settle by gravitation during the process of saturation with celloidin, and collected into a small area by a rotatory movement. On account of the minuteness of the objects thin sections are required and double-embedding is essential.

The ova are probably internally fertilised, but segmentation was never observed to begin until the egg had been laid.[1] The process of segmentation is complete but irregular. The first division (Pl. 23, fig. 1) is usually not quite median, one of the two cells formed being somewhat larger than the other. The extent of this difference is variable, and in one specimen of which I have sections the two cells are of equal size. One of the two cells, probably the smaller, when, as is the rule, they are unequal, divides into two by a longitudinal fissure. the other remaining undivided (Pl. 23, fig. 2). In the following divisions the latter cell does not take part, or takes part only to a slight extent; it retains the character of a single median cell, which, though it decreases in size and may have small segments cut off from it, yet greatly exceeds any of the rest in size, and occupies a large area at one of the poles. The pole in question may be regarded as the vegetal pole, and the large cell may be distinguished as the vegetal cell.

The divisions which follow upon the three-celled stage result in the attainment first of the four-celled (Pl. 23, figs. 3 and 4), then of the five-celled stage, which was met with several times (Pl. 23, figs. 5 and 6). The embryo now consists of the large plano-convex vegetal cell (*v.c.*) and four others, two median, unpaired, and two lateral. The latter are not symmetrical. One of them undergoes a special modification; it loses its nucleus and takes no further part in cell

[1] The first polar body is probably thrown off before fertilization, and thus before the shell is formed. Two rounded bodies which seem to represent the second occur along the first furrow. Later one is found about the animal pole.

division; its finely granular substance afterwards spreads out as a thin layer on the surface. This non-nucleated material or secondary yolk, as it may be termed, persists till a late period (see Pl. 23, figs. 13–18, *yk.*).

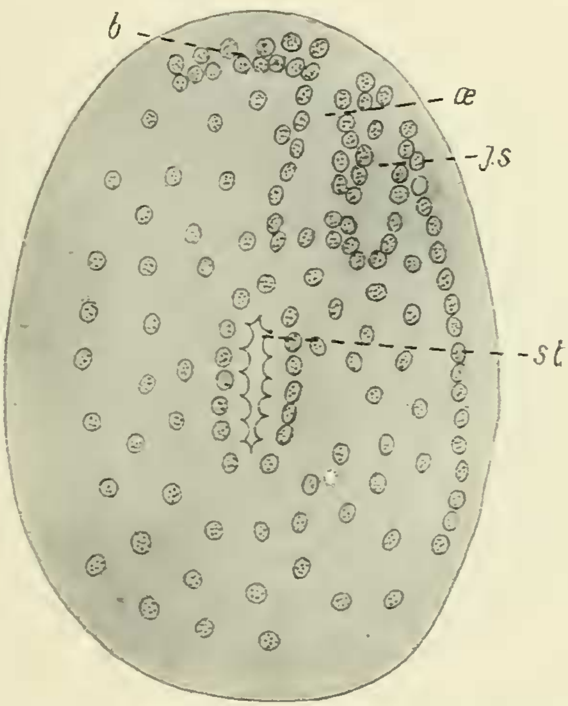
Further divisions follow, the vegetal cell still retaining its dominant position until when about fourteen cells have been formed (Pl. 23, fig. 9) the vegetal cell begins to sink inwards, the neighbouring smaller cells coming to encroach on its margin. About the same time it sends off a thick, rounded process towards the centre of the egg, and this becomes cut off by a fissure to form a separate, centrally-situated cell.

Meanwhile a change has begun which precludes the further following out of the history of the various elements.

The cells resulting from the segmentation process have up to this point been separated from one another by definite clean-cut fissures. But, at about the time when the sinking inwards of the vegetal cell takes place, this definite cell-division ceases, and complete fusion takes place among all the cells. The embryo now assumes the character of a quite regular, solid, oval mass with a few nuclei scattered through it without any trace of definite arrangement. Sections of this stage might readily be taken for sections of the unsegmented ovum, so complete is the homogeneity, were it not for the presence at irregular intervals of the small nuclei in various phases. Cell-division after this consists, so far as can be seen, simply of division of nuclei, and distinction between elements, on the ground of differences in their derivation, comes to be impossible.

In this undifferentiated body the first indications of certain structures are afforded by a mustering, multiplication, and arrangement of nuclei. In this way become established (Text-fig. 1) the first rudiments of the brain and nerve-cord, the œsophagus, pharynx, and stomach. Towards one end, the anterior, nuclei collect just below the surface and multiply to form what is at first an indefinite strand, but becomes a thick, dense plate of closely packed nuclei, the apical plate or rudiment of the brain (*b*). Along the opposite side also, just below the surface, extends a string of nuclei, which multiply to form ultimately a thick cord of densely aggregated nuclei similar to those forming the brain rudiment. The anterior end of this is separated by only a short interval from the apical plate, and it grows backwards round the blastoderm so

TEXT-FIG. 1.

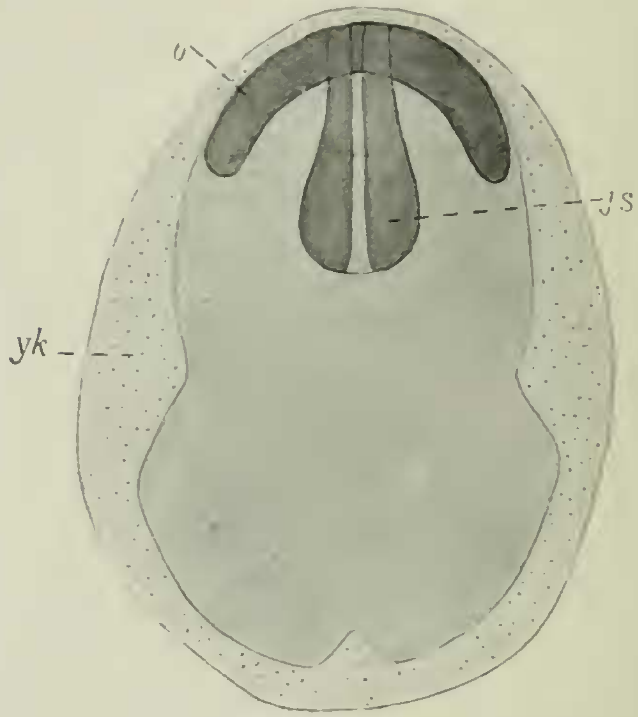


Sagittal section of stage in which the first rudiments of the brain, pharynx, and stomach have appeared. × 500. *b*. Brain. *j.s.* Jaw-sac. *œ*. Œsophagus. *st*. Stomach.

that its posterior end comes to lie almost in contact with the anterior end of the apical plate. It is to be noted that the rudiments of the apical plate and ventral nerve cord are formed at a stage when an ectoderm is not represented in any way, even by an arrangement of nuclei.

About the same time as the rudiments of the nervous those of the enteric system are developed, and as in the former case this involves at first nothing more than processes of multiplication and marshalling of nuclei. In the interspace between the rudiment of the apical plate and the anterior end of that of the nerve cord nuclei become arranged so as to circumscribe two spaces free from nuclei, though solidly filled with protoplasmic material. One of these represents (*œ*) the rudimentary œsophagus; the other (*j.s.*) the pharynx, or

Text-fig. 2.



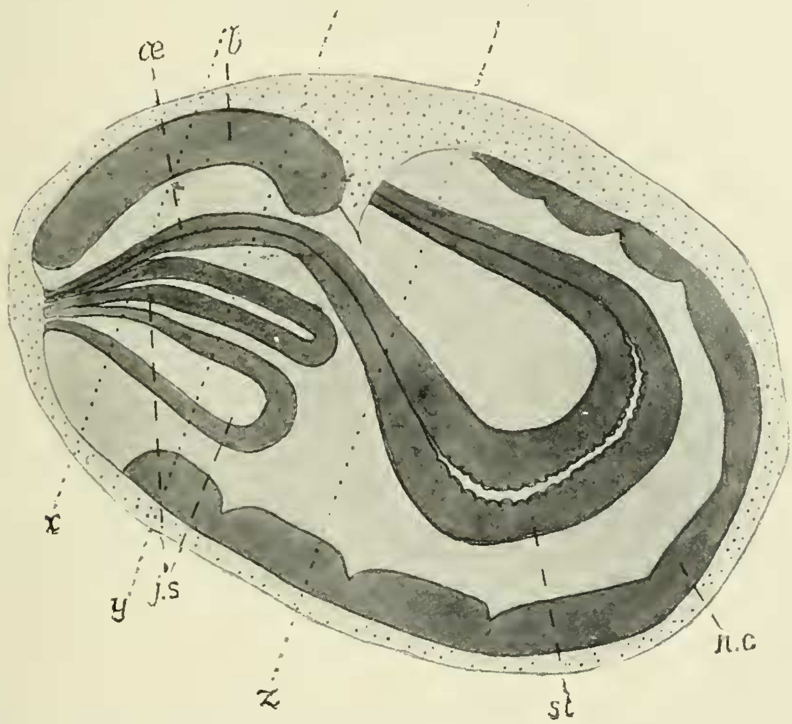
Early constricted stage viewed as a transparent object. × 600. Letters as in Text-fig. 1; in addition, *yk*. secondary yolk.

sac in which the jaws are destined to be developed; the former is nearer the brain rudiment, the latter near that of the nerve cord. At first they appear to be independent; later they unite externally so that, ultimately, they open by a common aperture, the mouth. Neither develops a lumen till a later stage.

Towards the middle of the embryo another enteric rudiment (*st.*), destined to give rise to the stomach-intestine, first appears during this stage before the constriction and curvature presently to be described have begun to alter the original oval shape. But in this case a lumen appears very soon, and the nucleated layer which surrounds it takes on an epithelial character from a very early stage.

Up to this point the embryo has retained the original elliptical form of the ovum. After the appearance of the rudiments of the nervous and enteric systems, certain changes

TEXT-FIG. 3.

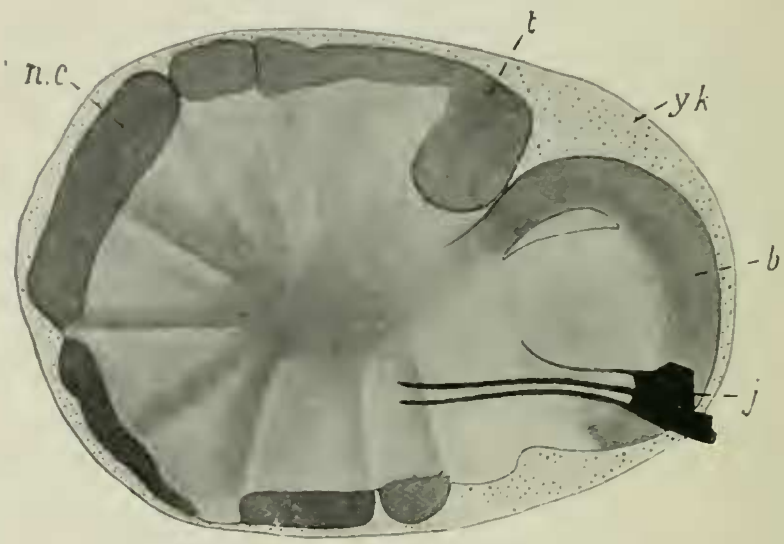


Sagittal section (semidiagrammatic) of stage just before the appearance of the jaws. × 600. Letters as in preceding figures; in addition, *n.c.* nerve cord. The dotted lines *x. y. z.* indicate the approximate planes of the sections represented in Plate 23, figs. 16, 17, 18.

of importance in the external configuration take place. The oval body undergoes a process of constriction at about the middle of its length resulting in its superficial separation into two parts which correspond respectively to the head and body-regions of later stages (Text-fig. 2). The former encloses the apical plate and the œsophageal and pharyngeal rudiments, the latter contains the ventral nerve cord and the stomach-intestine.

At the same time the embryo undergoes a process of dorsoventral flexure with the concavity on the dorsal side and the convexity on the ventral (Text-fig. 3). The deep bay on the dorsal side formed as a result of this process is occupied by the main bulk of the secondary yolk. The developing brain becomes bent over more definitely towards the dorsal side. As the embryo grows in length the curvature increases, and the head in its dorsal region comes into close apposition with the tail. Further growth results in the overlapping of the

TEXT-FIG. 4.



Lateral view of stage after completion of jaws, viewed as a transparent object. × 600. Letters as in preceding figures; in addition, *t*. tail.

regions, so that in the embryo ready to leave the egg the relations of the parts become complicated by a double process of folding, both head and tail being flexed on the trunk. Traces of segmentation appear as superficial transverse constrictions of the middle region soon after the primary dorsal ventral curvature has become well pronounced.

Around the developing intestinal epithelium is formed a thin layer with flattened nuclei. This thin layer, which is syncytial from the outset, is distinctly recognisable at a stage when no rudiment of the jaws has yet become visible. It becomes closely applied to the intestinal epithelium as the latter assumes its definite character, and is converted into the splanchnic layer of the cœlenchyme. The parietal layer of the latter with the muscular layer to which it gives rise are differentiated considerably later. Up to the time of hatching there is no cavity between these two layers; the cœlom must develop as a result of their separation at about the time of escape from the egg.

The stomach-intestine is at first straight, but, when the flexure occurs which leads to the differentiation of the dorsal surface, it becomes bent on itself in the form of a loop (Text-fig. 3). The anterior limb of this comes into connection with the œsophagus and opens into it; the posterior extends along the axis of the developing tail region and opens on the exterior at the posterior end. The apical plate or primitive brain becomes a thick mass of nuclei by active division. Later the neuropile is formed below this. The nerve cord is also at first represented only by a thick strand of nuclei. The ganglia are not distinctly marked off till a late stage. But the bilateral character of the cord is well marked long before any trace of the jaws has appeared.

The above-recorded observations on the embryonic development do not lead to any very definite conclusion as to the relationships of the Histriobdellidæ. Widely divergent opinions have been expressed on this subject, but the general consensus seems to admit, in the first place, that there is a fairly close connection between the Histriobdellidæ and the Dinophilidæ (Harmer, **4**, Pierantoni, **7**, Haswell, **5**, Shearer, **11**, Goodrich, **3**); and, in the second, that both these families present primitive, or degenerate, annulate characters.

Less widely maintained is the view (Haswell, **5**, Shearer, **11**) that there is a relationship of a fairly near character between the Histriobdellidæ, with Dinophilus, and the Rotifera.[1] With regard to the last question the observations above recorded, though tending somewhat in favour of this

[1] Schimkewitsch (**10**) admits this as a possibility in the case of Dinophilus.

conclusion, since there is quite striking similarity in the segmentation and gastrulation between the two groups, are disappointing as leading to the result that, owing to the syncytial condition which supervenes, the mesoderm cannot be traced to a derivation from any special cells.

With regard to the adult resemblances to Rotifers I may take the opportunity of pointing out two important difficulties to which attention has not yet been directed. It is easy to trace, as I have done (6) a correspondence between the parts of the mastax of the Rotifera and those of the jaws of the Histriobdellida; but such a comparison loses much of its force when we consider that the unpaired element or fulcrum is ventrally placed in the former case and dorsally placed in the latter. The second of the two points of difference which I now have in mind is that while in the male of the Rotifer the penis has a dorsal position, in the male of the Histriobdellid it is placed well forward on the ventral surface. But these differences, though they certainly add to the difficulty of co-ordinating the parts in the two groups, do not, I think, rule out the theory of a near connection of the Histriobdellida with the Rotifera, since, as regards the jaws, the proof of a precise correspondence of the chitinous pieces is not vital to the argument, and as regards the penis, in a form without enteric canal, a shifting from an original dorsal to a ventral position would not appear to be a change of a very radical character.

Summary.

(1) There is no metamorphosis.

(2) Segmentation is complete but unequal, and resembles closely the corresponding process in the Rotifera.

(3) At an early stage in segmentation one of the cells ceases to take part in the process of division and becomes converted into a mass of non-nucleated, finely granular material (secondary yolk), which remains distinct till a late stage.

(4) A large vegetal cell at one pole becomes immersed among the neighbouring cells, and the cells to which it gives rise in the interior of the embryo probably represent, in part at least, an endodermal layer.

(5) Complete coalescence subsequently takes place between all the cells of the embryo, this resulting in the formation of a syncytium in which the rudiments of organs first appear as a marshalling and multiplication of nuclei.

LITERATURE.

1. Beneden, P. J. van.—"Histoire naturelle d'un Animal nouveau designé sous le nom d'Histriobdella," 'Bull. Acad. Roy. Belg.' (2), tome v, 1858.
2. Car, Lazar.—"Die embryonale Entwickelung von Asplanchna Brightwellii." 'Biol. Centralbl.,' Bd. xix, 1899.
3. Goodrich, E. S.—Nerilla an Archiannelid," 'Quart. Journ. Micr Sci.,' N.S., vol. 57, 1912.
4. Harmer, S. F.—"Notes on the Anatomy of Dinophilus." 'Journ. Marine Biol. Assoc.,' N.S., vol. i.
5. Haswell, W. A.—"On a new Histriobdellid," "Quart. Journ. Micr. Sci.,' N.S., vol. 43, 1900.
6. ——— "Notes on the Histriobdellidæ," ibid., N.S., vol. 59, 1913.
7. Pierantoni, U.—"Protodrilus," 'Fauna u. Flora des Golfes v. Neapel,' 1908.
8. Salensky, W.—"Beitrage zur Entwickelungsgeschichte des Brachionus urceolaris," 'Zeitschs. f. wiss. Zool.,' Bd. xxii. 1872.
9. ——— "Ueber die Entwickelung von Brachionus urceolaris Entwickelung des Sommereies," 'Zeitschr. f. wiss. Zool.," Bd. xxii, 1872.
10. Schimkewitsch, W.—"Zur Kenntniss des Baues und der Entwickelung des Dinophilus vom. Wiessen Meere," ibid., Bd. lix, 1895.
11. Shearer, Cresswell.—"On the Anatomy of Histriobdella homari." 'Quart. Journ. Micr. Sci.,' N.S., vol. 55, 1910.
12. Tessin, G.—"Ueber Eibildung u. Entwickelung der Rotatorien" 'Zeitschr. wiss. Zool.,' Bd. xliv, 1886.
13. Zacharias, O.—"Ueber Fortpflanzung und Entwickelung von Rotifer vulgaris," ibid., Bd. xli, 1884.

14. Zelinka, C.—"Studien ueber Räderthiere, III, Zur Entwickelung," ibid., Bd. lii, 1891.

EXPLANATION OF PLATE 23,

Illustrating Mr. W. A. Haswell's paper "On the Embryology of Stratiodrilus (Histriobdellidæ)."

PLATE 23.

Fig. 1.—2-celled stage. × 600.

Fig. 2.—3-celled stage.

Fig. 3.—4-celled stage. From a mounted specimen.

Fig. 4.—4-celled stage. From an alcohol specimen.

Fig. 5.—5-celled stage.

Fig. 6.—Lateral view of the same stage.

Fig. 7.—6-celled stage.

Fig. 8.—Section (approximately saggital) of the same stage.

Fig. 9.—Stage of about 14 cells in which the large vegetal cell is beginning to become invaginated.

Figs. 10 to 12.—Sections through an embryo in which the invagination has proceeded further than in that represented in Fig. 9, the vegetal cell being now enclosed and having given off a daughter cell (*e*). Fig. 11 represents a section separated by two (not figured) from that represented in Fig. 10.

Figs. 13 and 14.—Two successive horizontal sections of an embryo at about the stage represented in Text-fig. 2; 13 more dorsal.

Fig. 15.—Section from a horizontal series of a stage somewhat later than that represented in Figs. 13 and 14.

Figs. 16–18.—Three sections of an embryo at about the stage represented in Text-fig. 3, in which the dotted lines x, y, and z indicate approximately the planes of section.